In fission yeast, Kinesin-5 also localizes to spindle poles, consistent with the hypothesis that similar localization is needed to optimally counterbalance forces by these two Klp families. Although the stoichiometry of proteins within γ-TuSC is known and conserved in eukaryotes little is understood of the γ-TuSC mechanism. Cross-species analysis is defining shared and distinct functions and novel insights into the γ-TuSC interactome and the role of associating Klps in regulating its mechanism for spindle assembly. Our studies apply site directed mutagenesis, genetics, yeast two hybrid, biochemistry, time-lapse microscopy and bioinformatic/structural approaches with novel reagents we have developed for Klp and γ-TuSC analysis.

670-Pos Board B470
Mitotic Kinesin Kar3Cik1 Interaction with Microtubules
Chun Ju Chen, Susan P. Gilbert.
Kar3Cik1 is a S. cerevisae Kinesin-14 motor protein that functions to shorten cytoplasmic microtubules (MTs) during yeast mating yet crosslinks interpolar MTs (ipMTs) during anaphase. Kar3 contains ATP and MT binding sites, yet Cik1 lacks the nucleotide binding site. Presteady-state kinetic and thermodynamic studies were pursued using paclitaxel-stabilized MTs to define the Kar3Cik1 interactions with the MT lattice expected during ipMT crosslinking. The results reveal that Kar3Cik1's association with the MT occurs at 4.9 μM-1s-1 followed by a 5 s-1 structural transition that limits mantADP release to 4-5 s-1. However, the intrinsic rate of mantADP release from Kar3Cik1 was observed at 109 s-1. ATP binding to nucleotide-free MT•Kar3Cik1 occurred at 2.1 μM-1s-1 followed by an ATP-promoted isomerization at 64 s-1. ATP hydrolysis was a fast step observed at 25 s-1, yet the reduced burst amplitude indicates reversals at this step. ATP hydrolysis was required for ATP-promoted Kar3Cik1 detachment from the MT at 12.7 s-1. The rate constant of phosphate release at 10 s-1 was similar to the rate constant of ATP-promoted MT•Kar3Cik1 dissociation, suggesting that these two steps are coupled. The rate-limiting step for steady-state ATP turnover at 5 s-1 is hypothesized to be the conformational change after Cik1 collision with the MT leading to Kar3 association with the MT followed by ADP release. These findings provide a model in which Kar3Cik1 interacts with the MT through an alternating cycle of Cik1 MT association followed by Kar3 binding with regulation through Kar3 nucleotide state and mechanical strain between the Kar3-Cik1 heads. Supported by NIH grant R01-GM54141 to SPG.

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Longitudinal and Rotational Motion of Microtubules Driven by the Kinesin-14 Motor Ncd
Andrej Vilfan, Bert Nitzsche, Elżbieta Kocik, Andrzej A. Kasprzak, Stefan Diez.
In gliding motility assays, various motor proteins have been shown to drive the rotation of filaments around their longitudinal axis. Using FLIC microscopy [1], we measured the longitudinal and rotational motion of quantum-dot labeled microtubules driven by Ncd (nonclaret disjunctional, a kinesin-14 motor protein) as a function of the ATP concentration. The data show two striking features: (i) The longitudinal velocity shows a strong deviation from the Michaelis-Menten curve. (ii) The rotational pitch depends strongly on the ATP concentration.
We propose a simple mechanical model which explains both findings. The underlying mechanism of the non-processive Ncd motor requires that the power stroke comprise a longitudinal, as well as a lateral (off-axis) component and that the waiting (apo) state take place before the power stroke. We note that the model is distinct from what has been proposed for actin rotation by myosin motors and microtubule rotation by kinesin-1 motors, where the rotation is a consequence of the filament structure [2, 1]. Our results are consistent with previous evidence based on cryo-EM data [3] that the Ncd power stroke is triggered by ATP binding.
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672-Pos Board B472
Reconstitution of Collective Transport by Defined Numbers of Kinesin-1 and Kinesin-14, Two Opposing Motor Proteins
Ken'ya Furuta, Yoko Y. Toyoshima, Hiroaki Kojima, Kazuhiro Oiwa.
Intracellular transport is usually driven by the collective operation of molecular motors that move along cytoskeletal filaments. Recent studies have reported that the movements include dynamic changes in velocity and direction along the filaments, which may be explained as a result of interplay between teams of opposing motors. In these movements, the number of participating motors is so small that its stochastic behavior dominates. Therefore, to reveal the mechanisms of motor coordination, it is essential to build and test mechanical models that explicitly incorporate the number and geometry of engaged motors. Here, we develop an experimental system to control the number and geometry of motors, i.e. 1, 2, 3, and 4 molecules, at specific sites on a single DNA scaffold. The covalent bonds throughout the whole complex allow us to precisely quantify the number of engaged molecules by SDS-PAGE, and ensure the stable linkages between motors for motion tracking and force measurement. First, we constructed complexes that engage teams of either kinesin-1 or kinesin-14. The travel distances of both kinesins were greatly extended when the number of molecules was increased, whereas the velocities were only slightly affected. Next, we linked together two opposing kinesin motors, kinesin-1 and kinesin-14, by a single molecular scaffold, which leads to a tug-of-war on microtubules. In the tug-of-war, reversals of direction and periodical, step-like movements were frequently observed. The combination of quantitative in vitro and in silico experiments using this simplified system will provide fundamental knowledge of motor coordination.

673-Pos Board B473
Biophysical Analysis of Microtubule Motor Traffic Jams
Leslie Conway, Derek Wood, Jennifer Ross.
The microtubule motor proteins, kinesin-1 and cytoplasmic dynein, are essential to transport cargo along microtubule tracks throughout the cell. This process relies on the processive nature of motor proteins. Single molecule studies show that both kinesin-1 and cytoplasmic dynein travel an average of 1 μm in distance with a velocity of ~0.6 μm/s *in vitro*. While these studies accurately depict the properties of these motors, they do not take into account the crowded environment these motors face *in vivo*. In contrast to dilute single molecule experiments, motors likely encounter numerous obstacles along the microtubule in cells. These obstacles could include other motors or microtubule associated proteins that bind along the microtubule, blocking the path needed by kinesin-1 or cytoplasmic dynein to continue their processive run. The objective of this study is to investigate how obstacles affect motor properties. We use increasing concentrations of the same or opposite motor to form obstacles *in vitro*. Motors are tagged with quantum dots to eliminate the issue of photobleaching and allow visualization of entire run lengths via total internal reflection fluorescence (TIRF) microscopy. Experiments are carried out using labeled motors in the presence of increasing concentrations of unlabeled motors. Processivity, velocity, dwell time, and pauses were measured for kinesin-1 across a concentration range of 1 nM-200 nM. We see that while velocity decreases in crowded conditions, the dwell time increases. Run length is seen to increase between 5 and 10 nM. In addition, pause frequency decreases in crowded conditions, while the average pause duration increases. We conclude that kinesin-1 motor properties are regulated by levels of crowding along the microtubule.

674-Pos Board B474
Deducing Kinetochore Protein Distributions along and Around a Microtubule Plus-End from FRET Measurements
Joshua Lawrimore, **Ajit Joglekar**.
The eukaryotic kinetochore is a sophisticated motor that moves and segregates chromosomes during cell division. As a microtubule-based motor, the kinetochore is distinct from all other motor proteins and microtubule-associated proteins: it attaches end-on to ~ 40 nm zone behind the plus-end of one or more microtubules, and it acts as a force coupler rather than an active force generator. These characteristics ensure that chromosome movement is tightly coupled with polymer growth and shortening at the plus-end. They also strongly suggest that kinetochore protein architecture, the distributions of the microtubule-binding kinetochore proteins along and around the microtubule plus-end and their dynamics, dictate the molecular mechanisms that generate movement. We have developed a FRET-based approach to determine the nanometer-scale distributions of multiple copies of the three principle microtubule-binding kinetochore complexes: Dam1, Ndc80, and Spc105. Sensitized emission measurements are carried out in metaphase and anaphase budding yeast (Saccharomyces cerevisiae) cells expressing kinetochore proteins labeled with Cerulean (donor) and Venus (acceptor) at desired locations. Preliminary measurements probing the distribution of the Ndc80 complex molecules show that the microtubule-binding head domains of adjacent Ndc80 molecules are ~ five nm apart, but this distance increases significantly at the other end of the molecule. The Dam1 complex is located more than 10 nm away from these head domains of Ndc80. We are developing Monte Carlo approaches to simulate the energy transfer processes among a known number of donor and acceptor molecules using known protein structures of some of the kinetochore proteins and the cylindrical microtubule lattice. These simulations will identify molecular distributions consistent with the FRET data, and illuminate the structural basis for microtubule end-coupled movement of the kinetochore.